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Abstract

The present work was designed to assess the modulatory effects of sesame oil (SO) and ascorbic acid (AA) on abamectin (ABM)-induced oxidative stress and altered gene expression of hepatic cytochrome P450 2E1 (CYP-2E1), p38 MAPK, and caspase-3 and cerebral P-glycoprotein (Abcb1a receptor). Male rats were distributed into five groups (6 rats/group), receiving distilled water, ABM 2 mg/kg bwt 1/5 LD_{50} orally for 5 days, ABM+AA 100 mg/kg bwt orally, ABM+SO 5 mL/kg bwt orally, or ABM+SO+AA at the aforementioned doses. Nineteen compounds were identified in the SO sample by GC-MS analysis, including tetradecane,2,6,10-trimethyl, octadecane, 1-hexadecanol,2-methyl, and octadecane,6-methyl. Abamectin significantly upregulated the hepatic CYP-2E1 expression with excess generation of oxidative radicals, as evident by the significant depletion of reduced glutathione and elevation of malondialdehyde concentration ($p \leq 0.05$) in rat liver and brain tissues. Further, ABM significantly increased TNF-α concentration, the expression of caspase-3 and p38 MAPK in the liver, as well as p-glycoprotein and GABA-A receptor in the brain. These results were in line with the observed histopathological changes. Sesame oil and/or AA supplementation alleviated ABM-induced cell damage by modulating all tested parameters. In conclusion, ABM induces oxidative stress and increases the expression of CYP-2E1, caspase-3, and p38 MAPK in the liver, as well as P-gp and GABA-A receptor in the brain. These effects could be ameliorated by SO and AA, alone and in combination, probably due to their anti-oxidant, anti-apoptotic, and gene-regulating activities.

Keywords: abamectin; oxidative stress; sesame oil; ascorbic acid; liver and brain; rats

Introduction

Pesticides are used worldwide to control pests, insects, and disease vectors in veterinary medicine and agriculture (Jones, 2018). Abamectin (ABM) belongs to the avermectins family; 16-membered macrocyclic lactones with a disaccharide substituent at the carbon-13 position. It is generated from soil actinomycete fungi (*Streptomyces avemitilis)* and is a combination of 80% avermectin B1a plus 20% avermectin B1b. Abamectin is used as an anthelmintic agent in cattle and sheep to eliminate gastrointestinal nematodes, lung worms and nasal bots (Campbell, 2012). Non-therapeutic exposures to ivermectin and other macrocyclic lactones may lead to toxic effects; only after oral ingestion of a large amount. Although the exact mechanisms remain unclear, macrocyclic lactones, when taken in large doses, may pass through the blood-brain barrier and produce GABA-mimetic toxic effects. In mammals, avermectin intoxication begins with hyperexcitability, incoordination, tremors, hypotension, and later develops into ataxia, coma, respiratory failure, and even death (Yang, 2012).

Avermectin is poorly metabolized in mammals; 80–98% of the initially administered dose gets eliminated in stool (Sun et al., 2005). The highest levels of ABM were detected in the liver and fat (owing to its lipophilic nature), while the lowest was found in the brain (Gonzalez Canga et al., 2009). Therefore, the detoxification of ABM may disturb the functions of hepatocytes. The biodistribution of ivermectin in the host depends on the efflux by P-glycoprotein (P-gp) and biotransformation by cytochromes P450 (Alberich et al., 2014). P-gp prevents brain toxicity by limiting the penetration of toxic compounds across the blood-brain barrier (Roulet et al., 2003). Besides, it contributes to the intestinal excretion of ivermectin (Ballent et al., 2006). Hepatic drug metabolism is attained by the microsomal cytochrome P450 enzyme system that facilitates transformation into toxic intermediates, followed by reactive oxygen species (ROS) production,

inflammatory cytokines release and lipid peroxidation (Lu et al., 2012; Maioli et al., 2013). These events initiate apoptosis and tissue inflammation through interaction with caspases and mitogen-activated protein kinases (MAPKs) (BayIr and Kagan, 2008).

Oxidative stress is a key factor in avermectins-induced cytotoxicity (Zhu et al., 2013). Therefore, the use of antioxidants to ameliorate its toxicity is a logical approach. Ascorbic acid is a water-soluble non-enzymatic antioxidant that defends the cellular compartments against reactive oxygen species (ROS) (Jurczuk et al., 2007). It is an essential element; must be supplied in diet because it cannot be synthesized in mammalian bodies. Ascorbic acid showed the ability to prevent lipid peroxidation and protect lipid membranes, proteins and DNA from oxidative harm (Granger and Eck, 2018). Moreover, it could antagonize the toxic effects of many xenobiotics (Abdel-Daim et al., 2019a; Abdel-Daim et al., 2019b; Özkan et al., 2012) and has been shown to renovate the antioxidant capacity of vitamin E (Serbecic and Beutelspacher, 2005).

Sesame oil (SO) is extracted from *Sesamum indicum* seeds. These seeds contain many phytochemicals as flavonoids, phenolic acids, tannins, alkaloids, terpenoids, cephalin and lecithin and some minerals like iron, calcium, magnesium, copper, zinc, manganese and phosphorus (Anilakumar et al., 2010; Sani et al., 2013). Several lignans, such as sesamolin, sesamin, and γ-tocopherol are potent polyphenolic antioxidants found in sesame seeds (Rangkadilok et al., 2010). For example, sesamin protects against oxidative stress and enhances hepatic drug detoxification (Shuang et al., 2018; Zhang et al., 2016a). Over decades of research, sesame (oil) exhibited anti-inflammatory, antibacterial, hypolipidemic, antitumor (Anilakumar et al., 2010) and anti-allergic effects (Jung et al., 2018).

The goals of this experiment were to explore the impact of acute exposure to the commercial formulation of ABM on the expression of metabolic cytochromes P450 2E1 (CYP-2E1), p38

MAPK and caspase-3 enzymes in the liver, as well as membrane p-glycoprotein efflux transporter and GABA-A signaling in rat brain. Further, we tested the preventive capacities of SO and/or AA against acute ABM toxicity.

2. Materials and methods

2.1. Chemicals: Abamectin *(Vertemic®*, 1.8% EC) was bought from *Syngenta Agro Services AG,* Egypt. Sesame Oil was supplied by *El-Captain* Company (El-Obour City, Egypt) and AA (*Ascorbin*® 100% B.p) was obtained from *Newvetrovit Company,* Egypt. Commercial kits for malondialdehyde (MDA), reduced glutathione (GSH) and catalase (CAT) were purchased from *Biodiagnostic Company,* Egypt. The kits for SGPT, SGOT, ALP enzymes, and direct bilirubin were supplied from *Greiner Diagnostic GmbH-Bahlingen, Germany*. The rest of used chemicals were of analytical grade. The chemical composition of sesame oil was analyzed using Trace GC-ISQ Mass Spectrometer (Thermo Scientific, Austin, TX, USA) as described in Supplementary file 1.

2.2. Experimental design: Thirty mature male albino rats (weighing 120 to 150 g), were purchased from the *Egyptian Company for Biological Products and Vaccines.* Animals were kept in stainless steel cages, fed rat chow and water *ad libitum*, and maintained at lab temperature of 25 ± 2 ^{o}C. All maintenance and care procedures were approved by the Research Ethical Committee at the Faculty of Veterinary Medicine, Beni-Suef University, Egypt.

After two weeks of acclimation, rats were randomly assigned to five groups (n = 6/group). The first group received distilled water (negative control). The second group (ABM) received a daily oral dose of ABM (2 mg/kg bwt, 1/5 LD_{50}) for 5 days (LD_{50}: 10 mg/kg) (Abdel-Daim and Abdellatief, 2018). The third group (ABM+AA) received ABM plus a daily dose of AA (100

mg/kg bwt, orally) (Abdel-Daim and El-Ghoneimy, 2015; Seo and Lee, 2002), while the fourth group (ABM+SO) was given ABM plus a daily dose of SO (5 ml/kg bwt, orally) (Saleem et al., 2014). The fifth group (Combination) received SO and AA with the same doses as before. Groups (3 to 5) were given SO and/ or AA for 10 days before ABM exposure and 1 hour before ABM administration for 5 successive days. Figure 1 summarizes the experimental design and observed findings.

2.3. Blood sampling and tissue processing: On the day following the last ABM dose, rats were anesthetized using isoflurane for blood withdrawal from the retro-orbital plexus, and then killed via cervical dislocation. The samples were centrifuged (at 3000 rpm for 15 min) to separate sera. The liver and brain were excised, washed with saline, and then blotted over filter paper. The tissue was divided into three parts: the first (0.5 g) was homogenized in phosphate buffer saline (pH 7.4, 5 ml). Homogenates were later centrifuged at 3000 rpm for 15 min at 4 ˚C using a cooling, high-speed centrifuge; supernatants were collected and preserved at −80 ˚C until analysis of tissue oxidant and antioxidant indices. The second tissue part was preserved at -80 ˚C for molecular investigations. The third tissue part was prepared for histopathological examination.

2.4. Biochemical estimations: The measurements of MDA, GSH concentrations and CAT activity were performed as per the methods described by Mihara and Uchiyama (Mihara and Uchiyama, 1978), Beutler et al. (Beutler et al., 1963) and Aebi (Aebi, 1984), respectively. Serum samples were used to measure the activities of SGPT and SGOT (Reitman and Frankel, 1957), ALP (Tietz et al., 1983), and bilirubin (Tolman and Rej, 1999).

2.5. Detection of TNF-α by ELISA: Tumor necrosis factor-α (TNF-α) levels were determined

144 by ELISA technique as per the methods of Tietz 1995 (Tietz, 1995). ELISA kits were purchased
145 from *R&D system* (MN, USA).

146 **2.6. Quantitative analysis of gene expression of CYP-2E1, Caspase-3, GABA-A receptor and**
147 **p-gp by real time PCR**

148 **Total RNA extraction:** Total RNA was extracted from the brain and liver tissue homogenates
149 according to the manufacturer's instruction, using *SV Total RNA Isolation System (Promega,*
150 *Madison, WI, USA*). The RNA concentrations and purity were measured with UV
151 spectrophotometer.

152 **Complementary DNA (cDNA) synthesis:** The extracted RNA was reverse-transcribed into
153 cDNA using high capacity cDNA reverse transcription kits (*#K1621, Fermentas, USA*) following
154 the manufacturer's instructions.

155 **Real-time quantitative PCR**: Real-time qPCR amplification and analysis were performed using
156 an Applied Biosystem with software version 3.1 (*StepOne™, USA*) to measure the expression of
157 mRNAs of target genes in the liver and brain, with *B-actin* as an internal reference (house-
158 keeping gene). The isolated cDNA was amplified using SYBR Green Master Mix (*Applied*
159 *Biosystems*) following the manufacturer's protocol. The primers used in the amplification are
160 shown in Table 1 and were designed by Gene Runner Software (*Hasting Software, Inc., Hasting,*
161 *NY*) from RNA sequences in the gene bank (based on published rat sequences). Data from real-
162 time assays were analyzed using the v1.7 sequence detection software from PE Biosystems
163 (*Foster City, CA*). Relative expression of the studied gene mRNA was calculated using the
164 comparative Cycle threshold (Ct) method. All values were normalized to β-actin and reported as
165 fold change over background levels detected in the treated groups. All these steps were
166 performed according to the methods of Livak and Schmittgen (Livak and Schmittgen, 2001).

2.7. Detection of p38 MAPK protein by Western Blot technique: p38 MAPK protein was detected following the manufacturer's protocol *(V3 Western Workflow™ Complete System, Bio-Rad® Hercules, CA)*. In brief, ice-cold radio immune precipitation assay (RIPA) buffer, along with phosphatase/protease inhibitors, were used for protein extraction from liver tissue homogenates. To visualize p38 MAPK protein, we used enhanced chemiluminescence (from *ECL plus; Amersham, IL*), followed by Molecular Analyst Software (*Bio-Rad, Richmond, CA*) for quantification. Protein levels were expressed in relation to β-actin.

2.8. Histopathological studies: Sections from the liver and brain tissues (M1 motor cortex and hippocampus) were fixed in buffered formalin, then stained with Hematoxylin & Eosin to examine pathological findings under a light microscope.

2.9. Statistical analysis: Data from the five experimental groups were summarized as means ± standard errors (SEM), and then transferred to a data sheet on the SPSS software (version 22, IBM Co., Armonk, NY). We used the ANOVA followed by Tukey's post-hoc test for experimental group comparison. P value ≤ 0.05 was accepted for significance.

3. Results

3.1. Sesame oil GC-MS analysis: In the present study, 19 compounds have been identified in the SO sample by GC-MS analysis (Table 2). The major components were tetradecane,2,6,10-trimethyl- (38.41%), octadecane (20.52%), 1-hexadecanol, 2-methyl- (9.14 %), octadecane, 6-methyl- (5.72%), 1-tetradecanol (3.85%) and 9-octadecenoic acid (Z)- (3.70%). The mass spectra of all major components in the studied sample are shown in Supplementary file 1.

3.2. Serum biochemical analysis: Oral administration of ABM was associated with significant ($p \leq 0.0001$) elevations in serum levels of hepatocyte injury biomarkers (SGOT and SGPT) and

biliary tract injury biomarkers (ALP and direct bilirubin), compared to the control group. Our results revealed the hepatoprotective effects of SO and AA, alone or in combination, as they significantly ameliorated the ABM-induced alterations in the four parameters. The obtained values in the ABM+SO and ABM+AA+SO combination groups were more frequently comparable to the control group, relative to the ABM+AA group (Table 3).

3.3. Hepatic oxidant/antioxidant markers: Acute ABM exposure was associated with significant ($p \leq 0.05$) increases in the hepatic tissue MDA concentration and CAT enzyme activity, as well as a significant drop in GSH level, compared to the control rats. Ascorbic acid and/or SO supplementation significantly ($p \leq 0.05$) restored MDA, CAT and GSH to the normal control levels. There were no significant variations among the treated groups (ABM+AA, ABM+SO or Combination group) (Table 4).

3.4. Brain oxidant/antioxidant markers: Our analysis showed a significant decrease ($p \leq 0.05$) in MDA concentration and significant increases in GSH content and CAT activity in the brain tissues of rats, treated with SO and/or AA, in comparison with ABM-exposed rats. Both treatments, either alone or in combination, could restore MDA and GSH to normal levels; however, the effect of combined treatment was more pronounced than that of a single treatment (Table 4).

3.5. Hepatic tumor necrosis factor-α concentration: Following ABM exposure, we observed significant elevations ($p \leq 0.05$) in liver tissue TNF-α concentrations in comparison to control rats. Treatments of ABM-intoxicated rats with SO, AA, or their combination was associated with significant reductions in hepatic TNF-α in comparison to rats, exposed to ABM alone. There were no significant variations among the treated groups (ABM+SO, ABM+AA or Combination group); Figure 2.

3.6. Expression of CYP-2E1, caspase-3, and p38 MAPK in the liver: Abamectin administration was associated with a significant boost in the hepatic tissue expression of CYP-2E1, caspase-3 and p38 MAPK proteins. In contrast, SO and/or AA supplementation ameliorated the ABM-induced increases in CYP-2E1, caspase-3, and p38 MAPK expression. There were no significant variations among the treated groups (ABM+SO, ABM+AA, or Combination groups). Figure 3 show the observed changes in CYP-2E1, caspase-3, and p38 MAPK expression in rats' liver tissues. Figure 4 shows the western blotting analysis of p38 MAPK hepatic expression in relation to β-actin.

3.7. Expression of ABC efflux transporter (*Abcb1a*) and GABA-A receptor in the brain: In this study, the expression of genes encoding the major ABC transporter (*Abcb1a*) was significantly increased following ABM exposure. This over-expression was significantly ameliorated in rats, treated with SO and AA, alone or in combination. Similarly, ABM exposure significantly upregulated GABA-A receptor expression in the brain of ABM-intoxicated rats (compared to normal controls), which was alleviated in rats treated with ABM plus SO, AA, or their combination (Figure 5).

3.8. Histopathological findings: Liver tissue sections from the control group show normal hepatic lobules with granulated and radiating hepatocytic cords from the central vein. However, tissue sections from ABM-exposed rats display lymphocytic infiltration and hemorrhage in the portal area, sinusoidal dilatation, as well as cellular hydropic degeneration, nuclear displacement, and focal ballooning of hepatocytes around the central vein. Marked reductions in these pathological alterations were observed after administration of SO and AA or their combination. Hepatic sections were nearly normal in the three treated groups except that those treated with SO and AA alone showed blood vessels congestion (Figure 6).

Cerebral cortex tissue sections from the control group show normal histological structure. In contrast, sections from ABM-intoxicated rats show dark stained nuclei, neuropil vaculation and hemorrhage. Rat groups, treated with SO or AA alone, showed marked reduction of hemorrhage, while the combination group sections show lightly-stained vesicular nuclei (Figure 7). Hippocampal tissue sections from the control group show normal outer pleomorphic, middle pyramidal, and inner molecular layers. However, sections from ABM-intoxicated rats show pyknosis, dark stained pyramidal neurons and degenerative changes in the middle pyramidal layer. Interestingly, ABM-intoxicated rats treated with SO only showed few pyknotic pyramidal cells, while rats treated with AA and SO+AA combination show nearly normal pyramidal cells with euchromatic nuclei (Figure 8).

4. Discussion

Oxidative stress is a key player in ABM-induced toxicity. The hemeprotein cytochrome P450 multi-enzymatic complex act mainly as mono-oxygenases for the metabolism of many compounds. During the metabolism of toxic substrates by CYP2E1, more reactive and toxic products are formed with excess generation of ROS (Danielson, 2002). These ROS further degrade the CYP hemeprotein to release iron, which catalyzes the Fenton's reaction, potentiating lipid peroxidation (Caro and Cederbaum, 2004). In the present work, the detected overexpression of hepatic CYP2E1 gene and resulting ROS probably played a role in the observed hepatic injury in ABM-intoxicated rats as indicated by the significant increases in the hepatic lipid peroxidation indicator (MDA concentration) and depletion of GSH stores in the liver. In addition, the brain redox markers were altered following ABM exposure.

Reduced glutathione is the principal antioxidant that removes ROS, generated by CYP2E1 (Chen and Cederbaum, 1998), thus GSH depletion (as observed in this study) leads to H_2O_2 accumulation, lipid peroxidation and cell damage. Catalase is a prominent endogenous antioxidant enzyme. The higher activity of hepatic CAT, noticed in the present study, may have been an adaptive response against H_2O_2 produced by ABM metabolism. In contrast, CAT activity was reduced in the brain of ABM-intoxicated rats. This result is parallel with that reported by Nasr et al. (Nasr et al., 2016), which reflects the failure of the brain antioxidant capacity to overcome ABM-induced oxidative stress, probably due to the high oxidative metabolism in the brain (Gandhi and Abramov, 2012).

The link between ROS and TNF-α is complicated; ROS increase TNF-α release and TNF-α increases ROS production (Blaser et al., 2016). Reactive oxygen species play as second messengers in the intracellular signal transduction pathways, including apoptosis. The p38 MAPK pathway is a series of serine/threonine kinases in mammalian cells, activated by excessive ROS generation (Di Lisa et al., 2011) and inflammatory cytokines (Segales et al., 2016). It mediates inflammatory response in various cell types by up-regulating TNF-*α,* interleukin-1 and interleukin-8 (Cuenda and Rousseau, 2007). In this study, ABM induced hepatocyte apoptosis, as confirmed by the increased caspase-3 expression in rat liver. These results are comparable to those detected in isolated rat hepatocytes (Maioli et al., 2013) and the pigeon liver (Zhu et al., 2013). The increased caspase-3 expression detected in our study may be related to the increased TNF-α level, which is involved in the extrinsic apoptotic pathway (Perez and White, 2000). Therefore, we suggest the possibility of ABM-induced apoptosis based on the extrinsic apoptotic pathway.

In addition, our results show that oral administration of ABM 1/5 LD50 (2 mg/kg bwt.) for 5 days significantly increased the serum activities of SGPT, SGOT and ALP in ABM-treated rats, compared to the control group. In parallel with prior investigations (Hsu et al., 2001; Khaldoun-Oularbi et al., 2013), these findings indicate that ABM causes permeability alteration and blood leakage of intracellular enzymes from the damaged hepatocytes. Oxidative stress and inflammatory cytokines activate heme-oxygenase-1 that regulates the synthesis of bilirubin (Yamamoto et al., 2007). Direct bilirubin is actively excreted at the canalicular membrane after binding to transporter proteins (Jedlitschky et al., 1997). Therefore, the damaged hepatocytes may be less able to produce the transporter proteins required for active transportation of direct bilirubin into the gall bladder, explaining the observed increase in its serum levels in the present study.

Because avermectins act as GABA-A receptor agonists in vertebrates, their safety in animals requires an intact blood brain barrier with integral P-gp. These are efflux transporters from the ATP-Binding Cassette (ABC) transporters superfamily (Jones and George, 2004), expressed within the capillary endothelial cells in the brain, placenta, and intestine (Ballent et al., 2006). P-gp protects animals against the diffusion of avermectins into the brain, avoiding the consequent neurotoxicity (Macdonald and Gledhill, 2007). In rodent genome, P-gp is encoded as two genes known as *MDR1a/Abcb1a* and *MDR1b /Abcb1b*. *Abcb1a* is more similar to the human gene and is the main form at the blood-brain barrier and intestine (Croop et al., 1989). In the current study, the expression of *Abcb1a* gene was significantly increased following ABM exposure. This finding is in accordance with a former study on ivermectin in murine hepatocytes (Ménez et al., 2012). In contrast, earlier studies showed that avermectin induces neurotoxicity via either oxidative damage, apoptosis (Li et al., 2013) or down regulation of P-gp (Sun et al., 2010).

303 The increased P-gp expression following ABM exposure in this study can be explained by
304 several mechanisms. Reactive oxygen species increase the hepatic expression of P-gp (Deng et
305 al., 2001) and brain endothelial cells in rats (Hong et al., 2006). Moreover, p38 MAPK can
306 activate NF-κB expression (Saha et al., 2007), which activates MDR1 gene transcription
307 (Bentires-Alj et al., 2003). Additionally, previous investigations have shown that up-regulation
308 of P-gp is stimulated by TNF-α in primary hepatocytes (Hirsch-Ernst et al., 1998) and the p38
309 MAPK pathway in rat prostate cancer cells (Sauvant et al., 2008). In the present study, despite
310 the detected overexpression of *Abcb1a* in the brain, the neurotoxic effect of ABM supports that it
311 is an effective inhibitor of p-gp facilitated transport (Lankas et al., 1998). Therefore, ABM could
312 increase *Abcb1a* expression as an adaptive response to oxidative stress. At the same time, ABM
313 inhibits the P-gp efflux transport function, increasing its penetration into the CNS and facilitating
314 the interaction with GABA receptors.

315 Another finding of this study is that ABM could significantly upregulate GABA-A receptor
316 expression in the cerebral tissue of ABM-intoxicated rats. The mechanism of action of
317 macrocyclic lactones-induced neurotoxicity (including ABM) in pests relies on their high affinity
318 for glutamate-gated Cl channels in neuronal and muscular cells. In mammals, they bind to the
319 receptor for the inhibitory neurotransmitter (GABA) and open the ionotropic GABA-A receptor-
320 gated Cl channels that are limited to the CNS (McCavera et al., 2007). Chloride ions then flow
321 into the postsynaptic neuron in excess, causing hyperpolarization of the membrane potential and
322 disrupting nerve signal transmission (Novelli et al., 2012).

323 Ascorbic acid and SO are commonly used as dietary supplements. Oral SO and/or AA
324 supplementation were able to inhibit the upregulation of CYP-2E1 expression in the liver of
325 treated rats. Similarly, sesamin reduced the expression of CYP-2E1 in hepatocytes (Zhang et al.,

2016b) and alleviated oxidative stress in acetaminophen or carbon tetrachloride-treated mouse liver (Ma et al., 2014). Previous studies have demonstrated that AA could directly react with lipid peroxides, increase GSH and antioxidant enzyme levels, and prevent resulting apoptosis (Santos et al., 2009; Wang et al., 2007). Our results showed that antioxidants like SO and AA eliminated ROS and lipid peroxides in the liver and brain tissues. Additionally, the effect of combined treatment was more pronounced in alleviating the oxidative stress in the brain tissue than that of a single treatment. This may be attributed to the augmenting effect of both treatments.

Administration of SO and AA significantly alleviated ABM-induced apoptosis by inhibiting the hepatic expression of caspase-3, as well as p38 MAPK and TNF-α. The anti-apoptotic activities of SO components and AA have been described in earlier studies. Ascorbic acid inhibited p38 MAPK phosphorylation in vitro (Carcamo et al., 2004). Similarly, Hou et al. reported that the inhibitory effect of sesamolin on caspase-3, p38 MAPK activation and ROS production could protect microglia against cell injury (Hou et al., 2004). Ma et al. found that CCl4-induced apoptosis was inhibited in liver by sesamin through reduction of hepatic TNF-α, Bak, Bax, Cyt. C and caspase-3 expression levels (Ma et al., 2014).

Sesame oil, AA, and their combination could ameliorate the significant increases in serum SGPT, SGOT and ALP, caused by ABM. These changes were more pronounced in combination group compared with that of single treated groups. Previous studies have shown that AA ameliorated the elevated serum SGPT and SGOT levels in rat models of malathion hepatotoxicity (Kalender et al., 2010) and organophosphate pesticide toxicity (Ambali et al., 2007). Further, SO ameliorated subacute diazinon toxicity (Abdel-Daim et al., 2016) and protected brain cells against cypermethrin toxicity (Hussien et al., 2013). Sesame oil

significantly alleviated ABM-induced elevation serum direct bilirubin, as observed previously in diazinon intoxicated rats (Al-Attar et al., 2017). These effects may be explained in the light of GC-MS analysis findings; several compounds within the analyzed SO sample, such as octadecane and 9-octadecenoic acid have anti-inflammatory activities and have been shown to mitigate oxidative stress and inhibit the formation of arachidonic acid.

In this study, oral SO and/or AA supplementation counteracted the ABM-induced up-regulation of *Abcb1a* expression. Previous research has proven the ability of flavonoids to inhibit P-gp transporters via inhibition of ATPase activity (Pulido et al., 2006) or competitive inhibition (Alvarez et al., 2010). Thus, the phenolic sesamin, sesamol, and flavonoids in SO may be responsible for these effects concerning *Abcb1a* gene (Anilakumar et al., 2010). Further, SO and AA alleviated ABM-induced overexpression of GABA-A receptor in rat brain. The brain is susceptible to oxidative injury due to its high oxygen consumption and a high content of polyunsaturated fatty acids (Gandhi and Abramov, 2012). Redox agents can regulate the GABA-A receptors function (Calero and Calvo, 2008)**.** In contrast, AA has been shown able to control the activity of glutamate and GABA receptors to protect neurons against glutamate excitotoxicity (Calero et al., 2011)**.** Therefore, in the present study, the simultaneous administration of SO and AA could significantly modulate GABA-A receptor mRNA levels owing to their synergistic ROS-scavenging effect.

In conclusion, ABM induced oxidative stress and increases the expression of TNF-α, caspase-3 and p38 MAPK. Moreover, it upregulates the expression of drug detoxifying genes; the brain *Abcb1a* efflux transporter and the hepatic CYP-2E1 enzyme. However, pretreatments with SO and AA effectively ameliorated ABM-induced oxidative stress and apoptosis. Simultaneous

371 administration of SO and AA was more efficient in protecting the rat liver and brain than single
372 agent use.

373 **Conflicts of Interest:** All authors declare no conflicts of interest

374 **Acknowledgement:** This project was supported King Saud University, Deanship of Scientific
375 Research, College of Science Research Center.

376

377 **Abbreviation:** AA: Ascorbic acid, ABM: Abamectin, ALP: Alkaline phosphatase, CAT:
378 Catalase, CYP-2E1: Cytochrome P-450 2E1, GABA: Gamma aminobutyric acid, GSH: Reduced
379 glutathione, MDA: Malondialdehyde, MAPK: Mitogen-activated protein kinases, P-gp: P-
380 glycoprotein, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic
381 transaminase, SO: Sesame oil, TNF-α: Tumor necrosis factor-α

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586

587

588 **Figure Legends**

589 **Figure 1:** Synthesis of the experimental design along with assessed molecular mechanisms. AA:
590 Ascorbic acid, ABM: Abamectin, CAT: Catalase, CYP-2E1: Cytochrome P450 2E1, GSH:
591 Reduced glutathione, MDA: Malondialdehyde, ROS: reactive oxygen species, SO: Sesame oil,
592 TNF-α: Tumor necrosis factor-α

593 **Figure 2:** The hepatic concentration of tumor necrosis factor-α (pg/mg) in different experimental
594 groups. AA: Ascorbic acid, SO: Sesame oil. Columns represent means ± SEM (n=6). Columns
595 with different superscript letters are significantly different at $p \leq 0.05$.

596 **Figure 3:** Expression of cytochrome P450-2E1 (CYP-2E1), caspase-3, and p38 MAPK proteins
597 in the liver of different experimental groups (in relation to β-actin). AA: Ascorbic acid, SO:
598 Sesame oil. Columns represent means ± SEM (n=6). Columns with different superscript letters
599 are significantly different at $p \leq 0.05$.

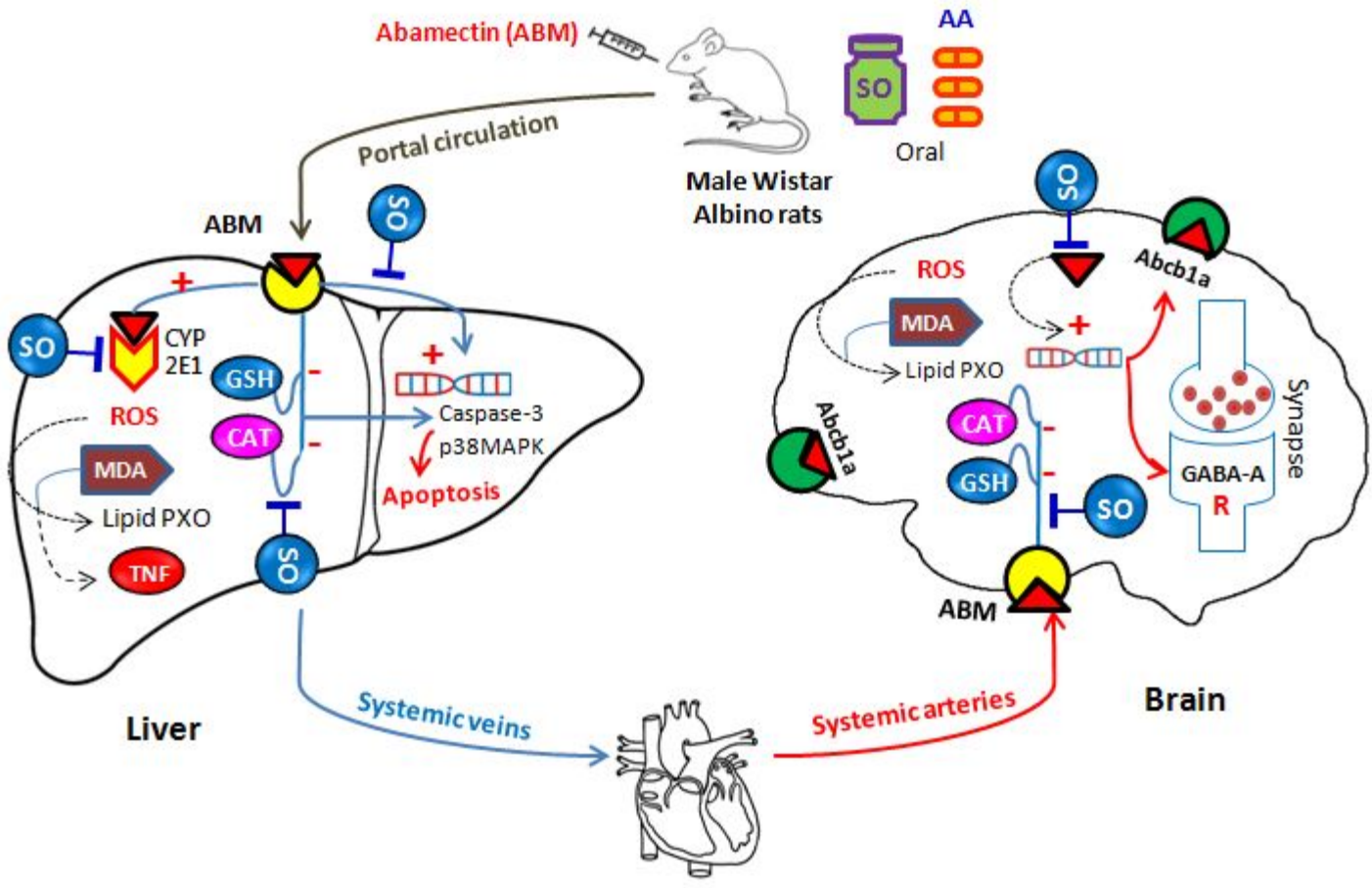
600 **Figure 4:** Western blotting analysis of p38 MAPK hepatic expression (in relation to β-actin) in
601 different experimental groups.

602 **Figure 5:** Relative expression of brain P-glycoprotein (*Abcb1a*) in different groups and GABA-
603 A receptor in different groups. AA: Ascorbic acid, SO: Sesame oil. Columns represent means ±
604 SEM (n=6). Columns with different superscript letters are significantly different at $p \leq 0.05$.

605 **Figure 6:** Photomicrographs of the hepatic sections stained with H&E. (A) Control group
606 showed normal structure, (B) ABM group showed lymphocytic infiltration (*) and hydropic
607 degeneration (curved arrow). The incite showed ballooning of hepatocytes (arrow). The treated
608 groups with SO (C) and AA (D) showed congested bold vessels (thick arrow). (E) The
609 combination group was nearly normal. Scale bar 50 um. Incite sale bar 20 um.

Figure 7: Representative photomicrographs of cerebral cortices of experimental groups stained with H&E. (A) Control group showing normal histological structure. (B) ABM group showing dark stained nuclei (arrow), neuropil vacuolation (arrowhead) and hemorrhage (*). Rats treated with SO (C) and AA (D) showing marked reduction of hemorrhage and (E) Combination showing lightly stained vesicular nuclei (arrow). Scale bar 50 um.

Figure 8: Representative photomicrographs of hippocampus (CA3) of experimental groups stained with H&E. (A) Control group showing outer pleomorphic layer (o), middle pyramidal layer (p) and inner molecular layer (M). (B) Abamectin group showed pyknosis, dark stained pyramidal neurons (arrow) and degenerative changes in the middle pyramidal layer. (C) treated with SO only few pyknotic pyramidal cells (arrow). (D) Rats treated with AA and (E) SO + AA combination showing nearly normal pyramidal cells with euchromatic nuclei (arrow). Scale bar 50 um.


Abamectin (ABM)
AA
SO
Oral
Male Wistar
Albino rats
Portal circulation
ABM
OS
SO
CYP
2E1
ROS
MDA
Lipid PXO
TNF
GSH
CAT
Caspase-3
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Apoptosis
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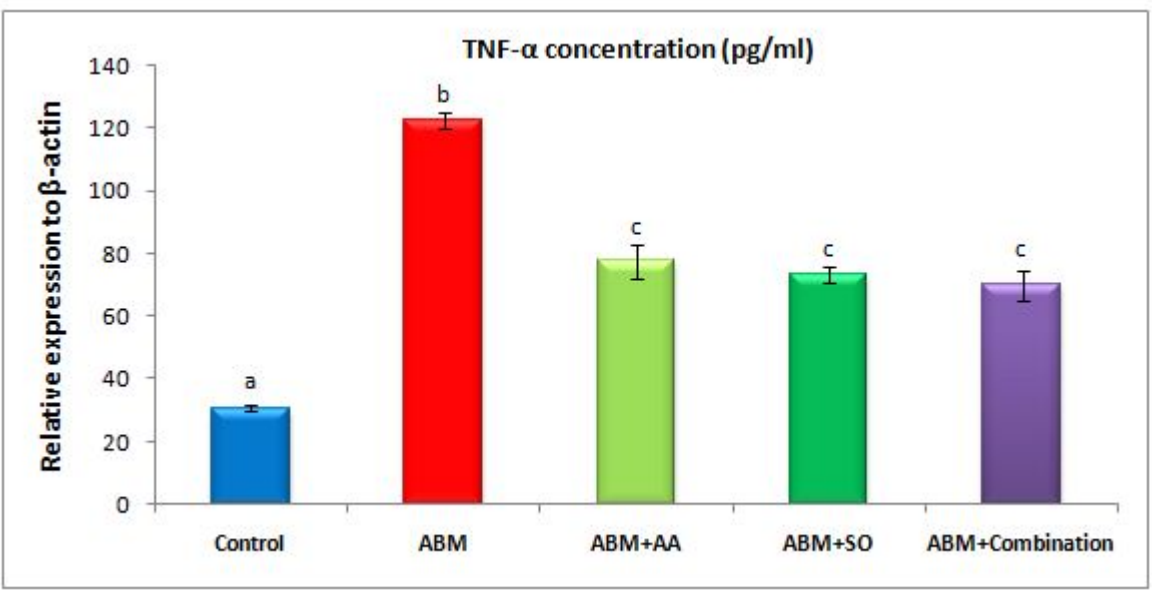
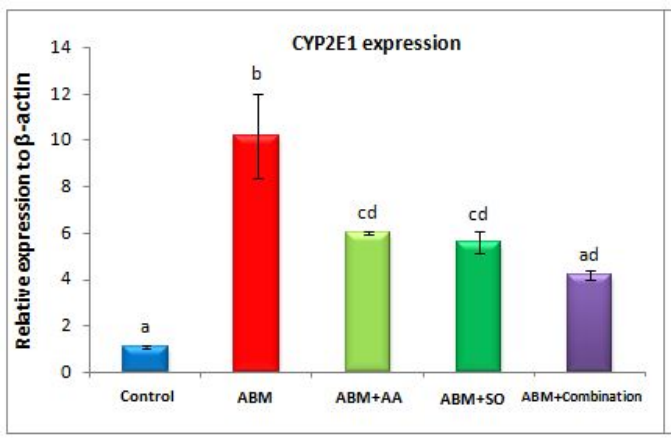
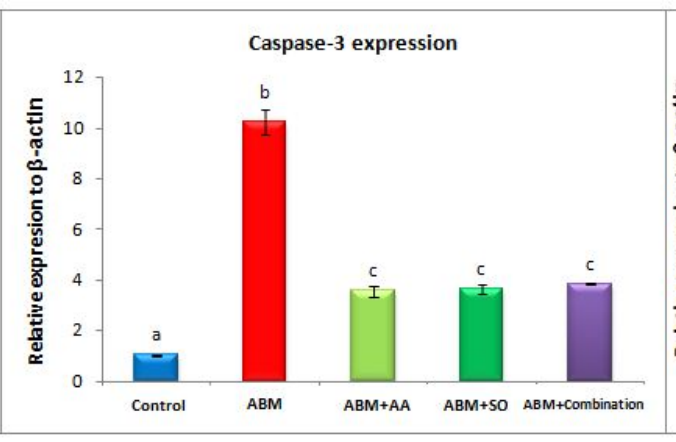
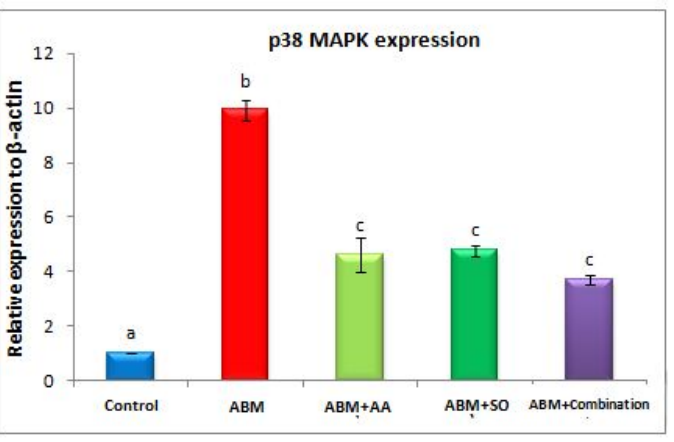
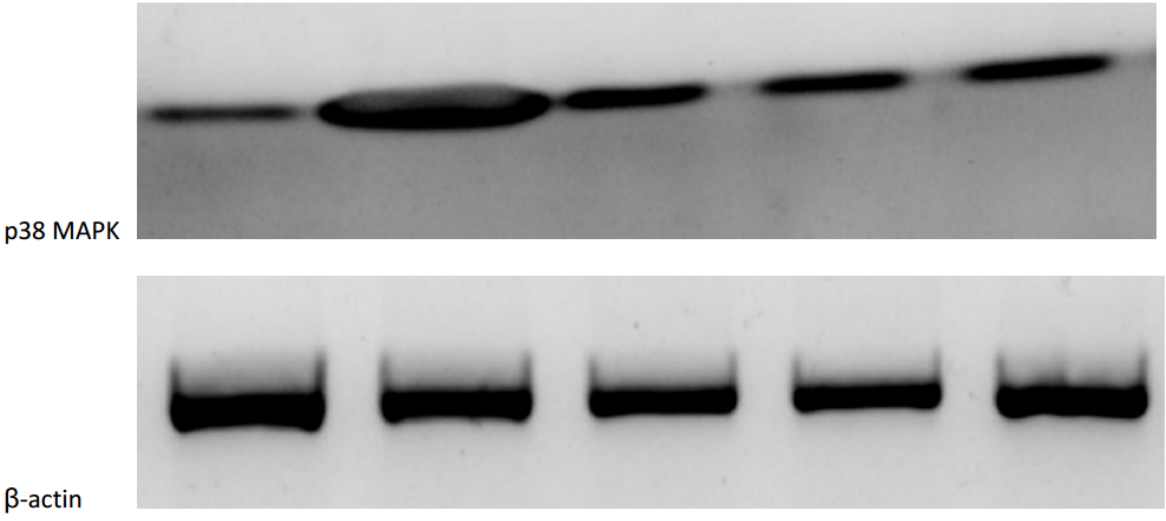
Brain
Abcb1a
Abcb1a
Synapse
GABA-A
R

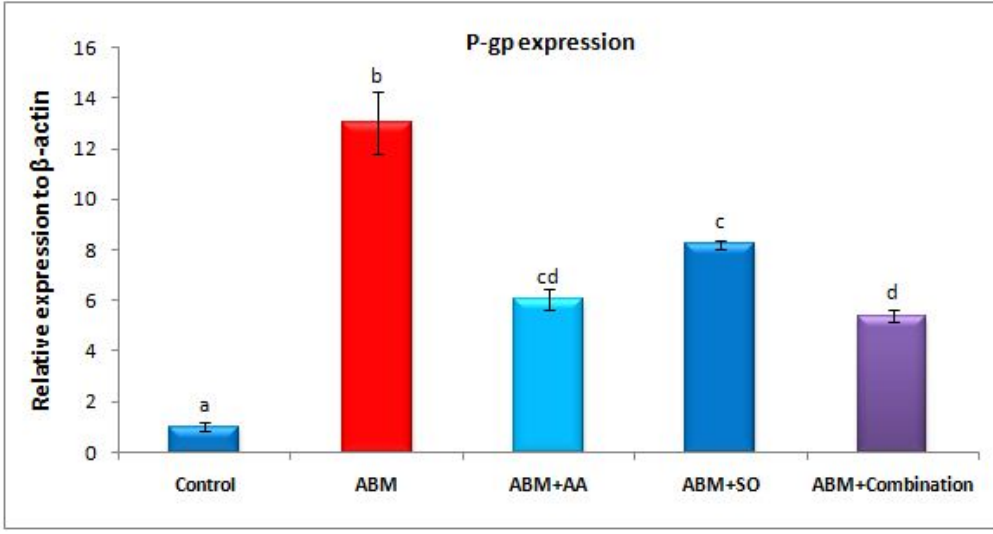

TNF-α concentration (pg/ml)
Relative expression to β-actin
140
120
100
80
60
40
20
0
a
b
c
c
c
Control
ABM
ABM+AA
ABM+SO
ABM+Combination

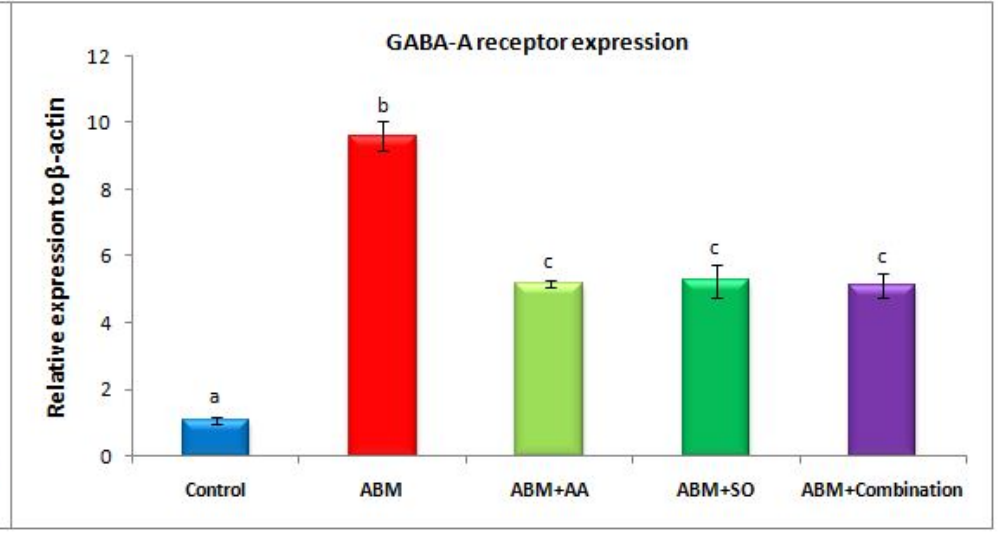

CYP2E1 expression
Relative expression to β-actin
14
12
10
8
6
4
2
0
a
b
cd
cd
ad
Control
ABM
ABM+AA
ABM+SO
ABM+Combination

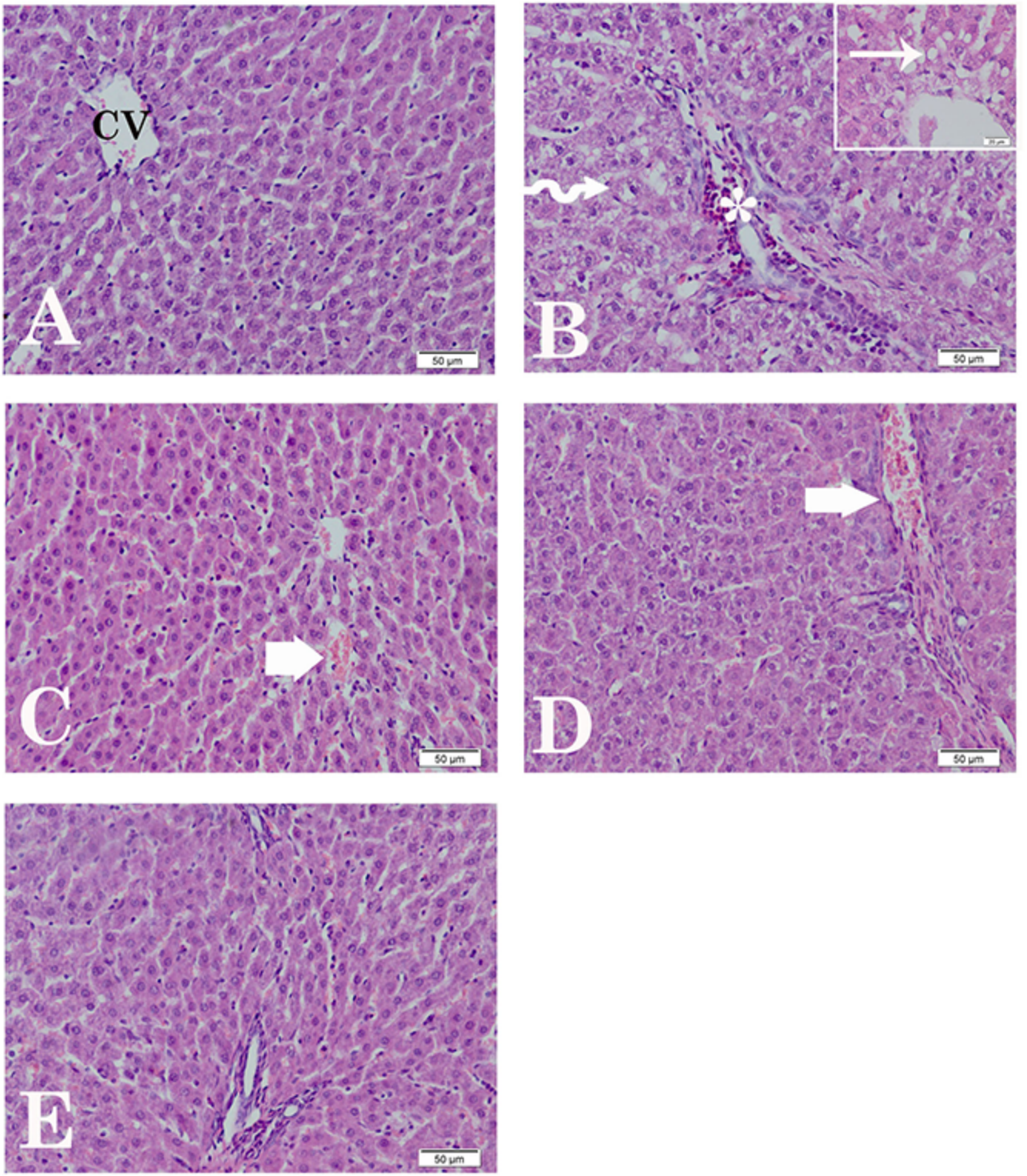

Caspase-3 expression
Relative expression to β-actin
12
10
8
6
4
2
0
a
b
c
c
c
Control
ABM
ABM+AA
ABM+SO
ABM+Combination

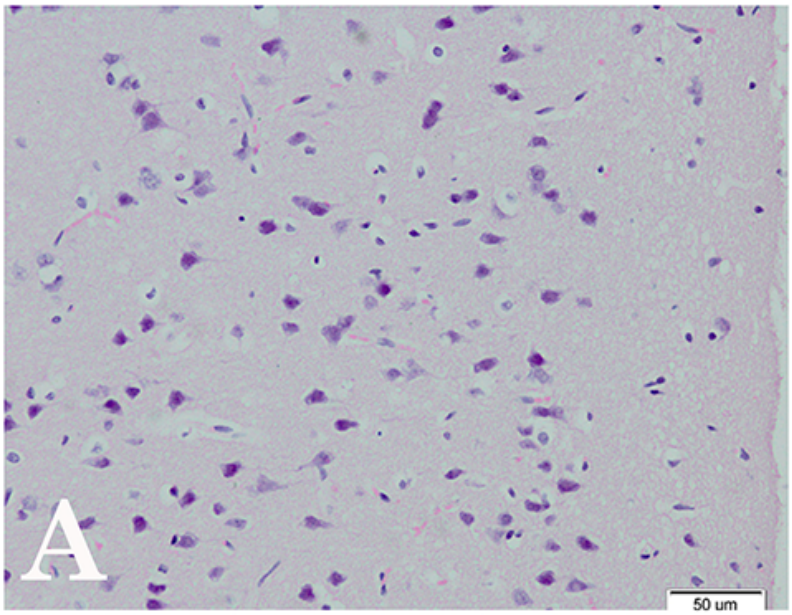

p38 MAPK expression
Relative expression to β-actin
12
10
8
6
4
2
0
a
b
c
c
c
Control
ABM
ABM+AA
ABM+SO
ABM+Combination

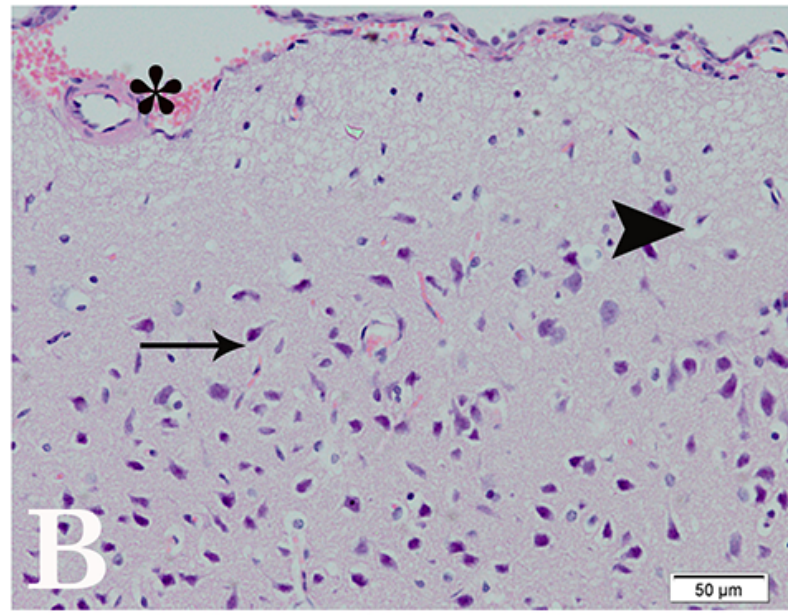

p38 MAPK
β-actin
Control
ABM
ABM+Vit.C
ABM+SO
ABM+AA+SO

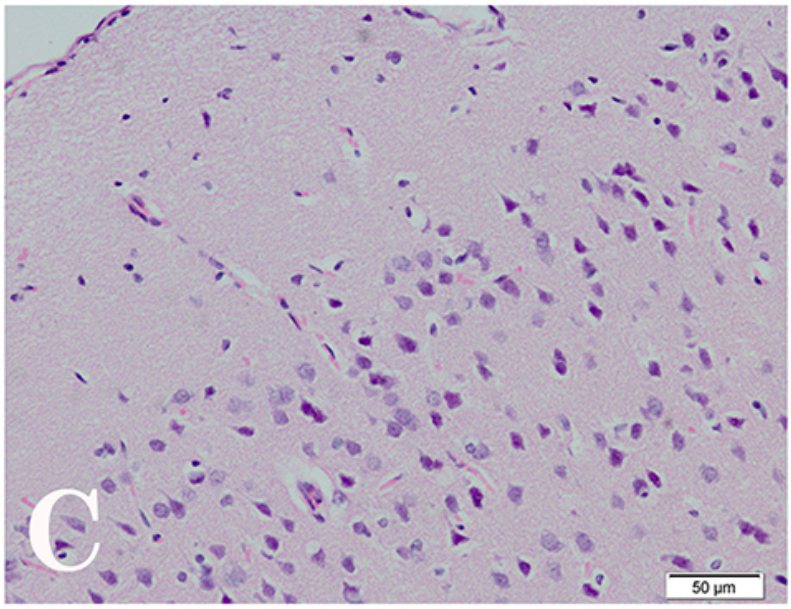

P-gp expression
Relative expression to β-actin
16
14
12
10
8
6
4
2
0
a
b
cd
c
d
Control
ABM
ABM+AA
ABM+SO
ABM+Combination

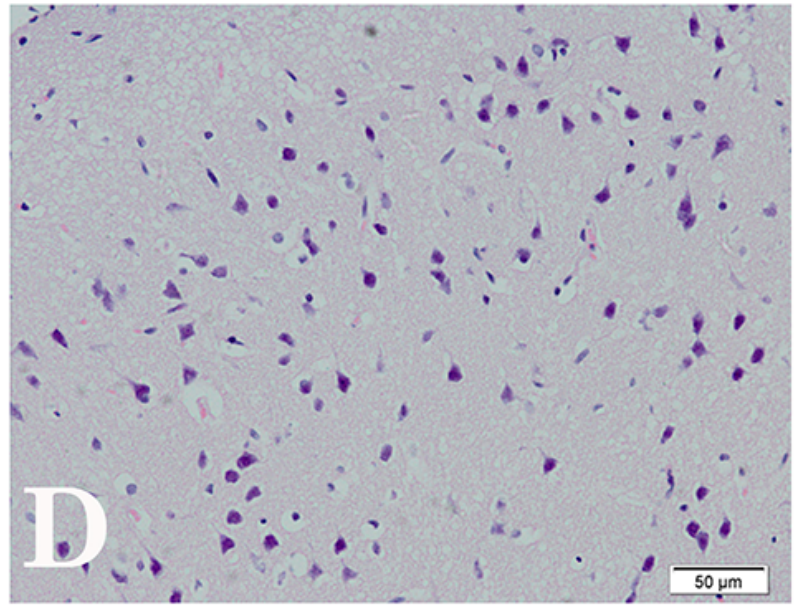

GABA-A receptor expression
Relative expression to β-actin
12
10
8
6
4
2
0
a
b
c
c
c
Control
ABM
ABM+AA
ABM+SO
ABM+Combination

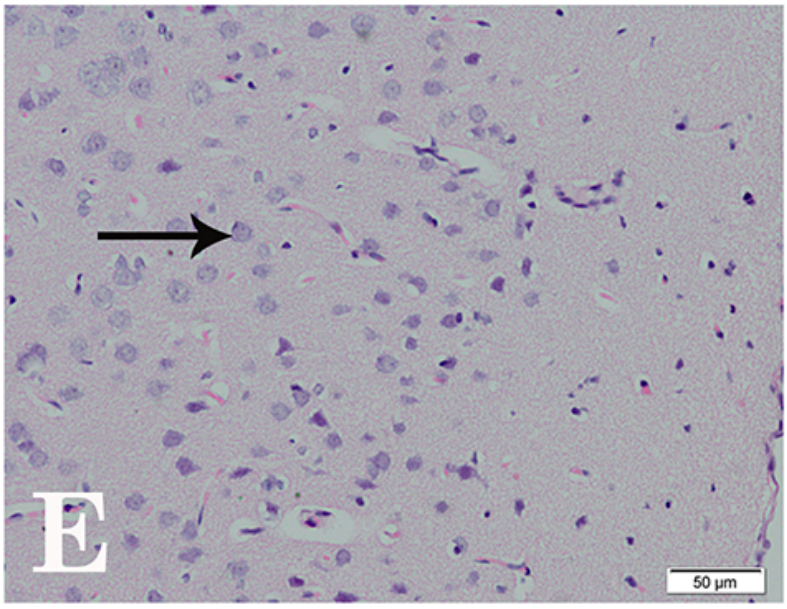

CV
A
50 µm
B
50 µm
C
50 µm
D
50 µm
E
50 µm


A
50 um


B
50 µm


C
50 µm


D
50 µm


E
50 µm

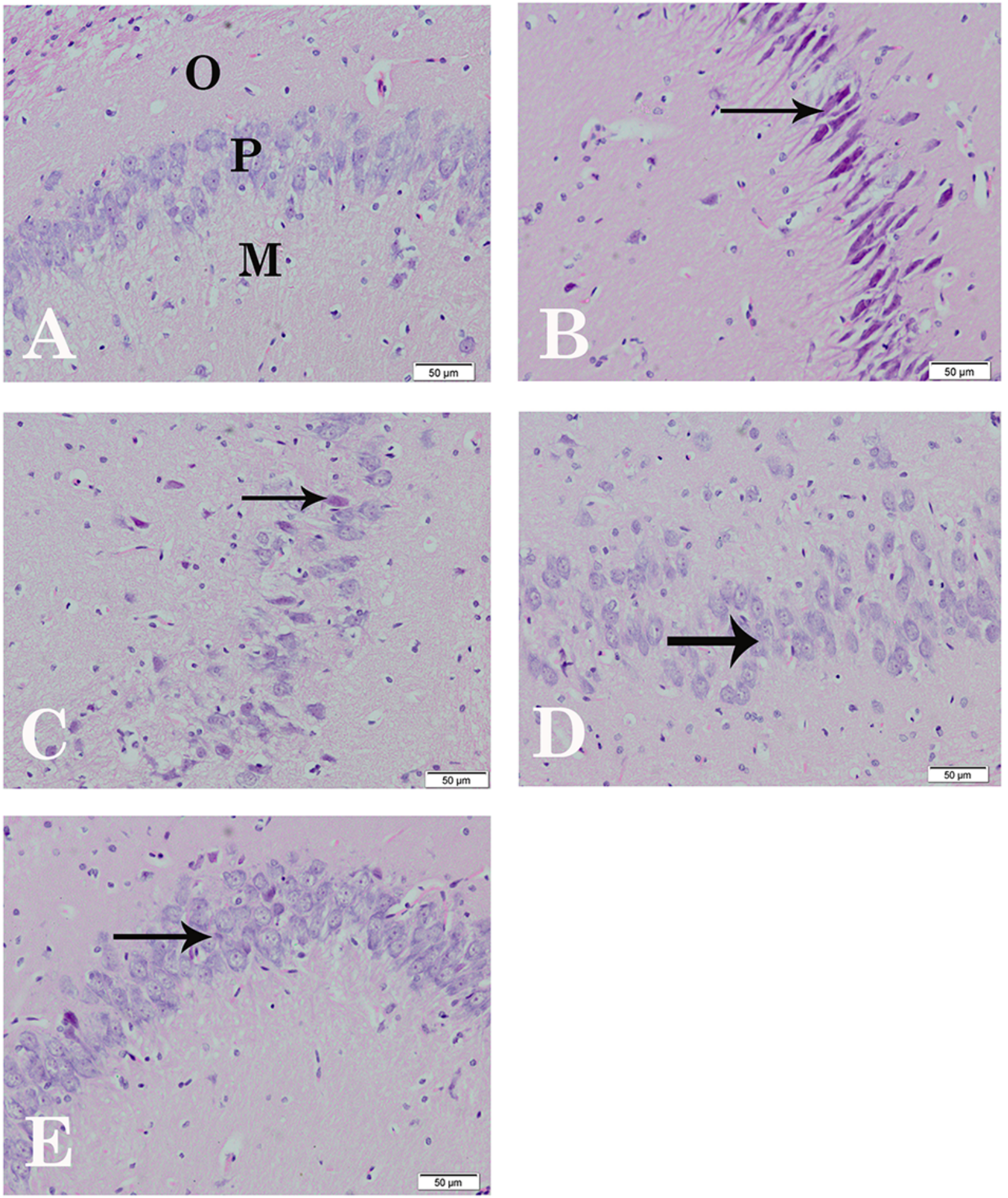

O
P
M
A
50 µm
B
50 µm
C
50 µm
D
50 µm
E
50 µm

Table (1): The primer sequence of the studied rat genes

	Primer sequence
GABA	Forward primer: 5'- TCGGGACCAACCCAACGTGC -3 Reverse primer: 5'- CGTGC TGGCCTGATTGACGCT -3
Abcb1a	Forward primer: 5-ACCAGCGGTCAGTGTGCT-3 Reverse primer: 5-CGGTTGTTTCCTACATTTGC-3
Cytochrome P450 2E1	Forward: 5- TTTGGATCCAATGGGTGATGTTGAG -3 Reverse: 5- TTTGAATTCCTCATTAGTAGCTTTTTTGAG-3
Caspase-3	Forward primer: 5'- TTC ATT ATT CAG GCC TGC CGA GG -3 Reverse primer: 5'- TTC TGA CAG GCC ATG TCA TCC TCA -3
B-actin	Forward primer: 5'--GGTCGGTGTGAACGGATTTGG -3 Reverse primer: 5'- ATGTAGGCCATGAGGTCCACC-3

Table 2: The chemical composition of sesame oil sample by gas chromatography-mass spectrometry

No.	RT	Name of the compound	Molecular Formula	Molecular weight	Peak Area %
1	3.36	Nonanol	C9H20O	144	0.40
2	11.50	1-Tetradecanol	C14H30O	214	3.85
3	13.30	Undecanal	C11H22O	170	0.49
4	14.49	Dodecane	C12H26	170	0.59
5	15.93	1-Hexadecanol, 2-methyl-	C17H36O	256	9.14
6	16.25	Nonadecane	C19H40	268	2.64
7	16.49	Tetradecane, 2,6,10-trimethyl-	C17H36	240	38.41
8	18.19	12-Methyl-E,E-2,13-octadecadien-1	C19H36O	280	1.81
9	18.68	Octadecane, 6-methyl-	C19H40	268	5.72
10	20.57	Z-8-Methyl-9-tetradecenoic acid	C15H28O2	240	0.96
11	21.36	1-Dodecanol, 3,7,11-trimethyl-	C15H32O	228	3.14
12	22.24	9-Octadecenoic Acid (Z)-	C18H34O2	282	3.70
13	22.44	Hexadecadienoic Acid, methyl ester	C17H30O2	266	1.29
14	22.84	Docosane	C22H46	310	3.01
15	23.11	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	C19H34O2	294	0.72
16	23.55	2-Dodecen-1-yl(-)succinic anhydride	C16H26O3	266	1.77
17	25.91	9-Hexadecenoic acid	C16H30O2	254	0.51
18	28.16	7-Methyl-Z-tetradecen-1-ol acetate	C17H32O2	268	0.67
19	28.64	Octadecane	C18H38	254	20.52

Table (3): Changes in serum SGPT, SGOT, ALP and direct bilirubin concentrations in different groups

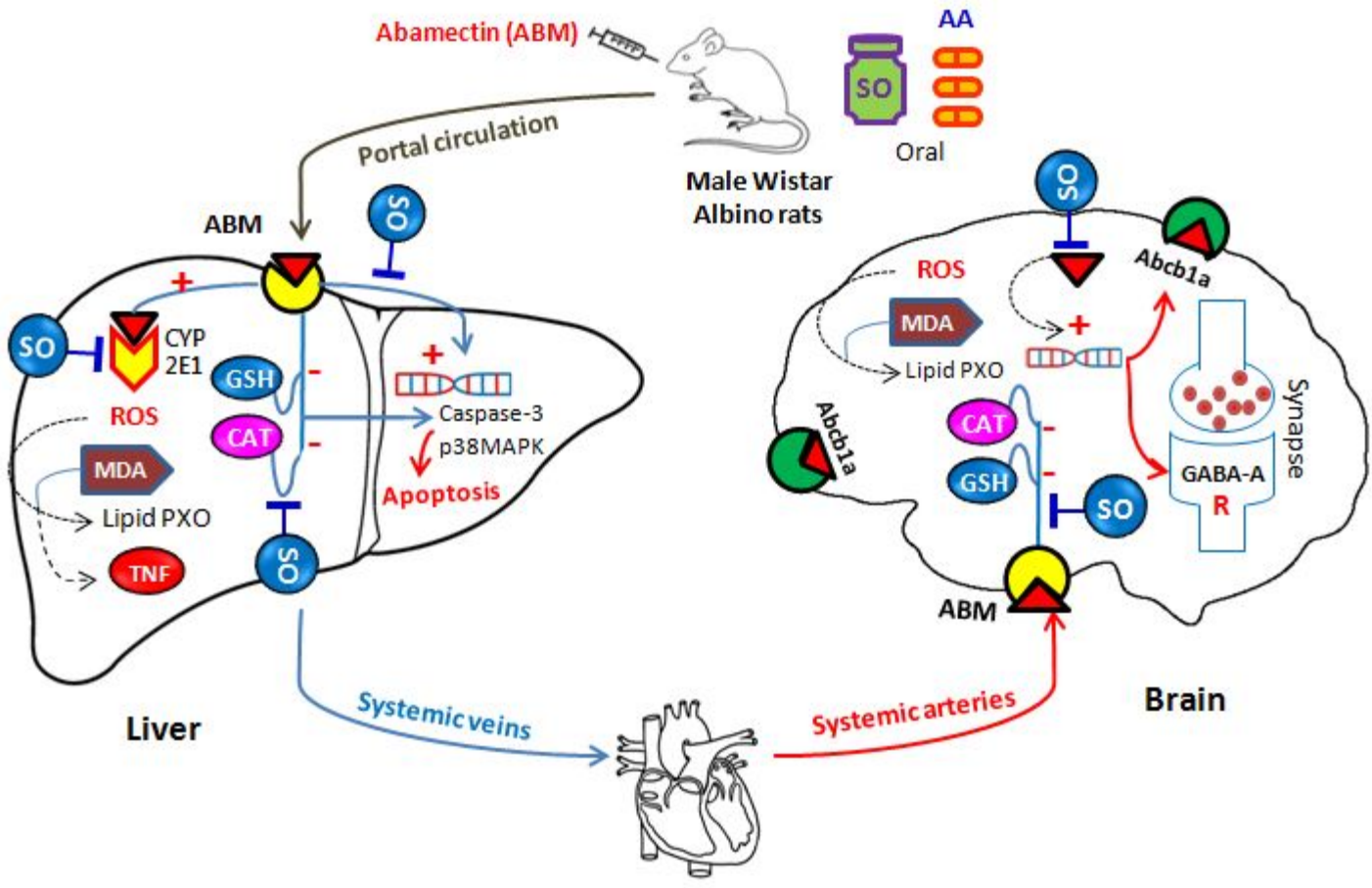
	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	Direct bilirubin (mg/dl)
Control	62.27±2.82[a]	58.03±4.49[a]	68.58±3.92[a]	0.99±0.05[a]
ABM	79.46±1.88[b]	86.87±4.55[b]	125.6±6.03[b]	2.09±0.10[b]
ABM+AA	67.16±0.76[a]	76.30±2.13[c]	91.08±3.74[c]	1.64±0.04[c]
ABM+SO	68.23±1.77[a]	70.72±3.41[ac]	84.67±3.13[ac]	1.43±0.03[c]
ABM+AA+SO	68.83±0.49[a]	67.10±2.62[ac]	80.21±1.94[ac]	1.40±0.06[c]

Values are expressed as mean ± SEM (n = 6 per group). Values with different letters in a column are significantly different at level $p < 0.0001$. AA: Ascorbic acid, ABM: Abamectin, ALP: Alkaline phosphatase, SGPT: Serum glutamic pyruvic transaminase, SGOT: Serum glutamic oxaloacetic transaminase, SO: Sesame oil

Table (4): Changes in MDA, GSH concentrations and catalase activity in the brain and liver of rats in different groups

	MDA (n.mol/g tissue)		GSH (mg/g tissue)		Catalase (U/g tissue)	
	Brain	**Liver**	**Brain**	**Liver**	**Brain**	**Liver**
Control	10.84±0.24^{a}	15.25±0.91^{a}	16±0.12^{ac}	16.24±0.12^{a}	0.634±0.01^{a}	0.236±0.01^{a}
ABM	13.20±0.37^{b}	18.63±0.49^{b}	14.12±0.23^{b}	12.22±0.79^{b}	0.461±0.04^{b}	0.323±0.01^{b}
ABM+AA	11.86±0.57^{ab}	15.19±0.32^{a}	15.61±0.23^{a}	15.79±0.06^{a}	0.624±0.01^{a}	0.303±0.01^{bc}
ABM+SO	11.51±0.52^{ab}	15.08±0.51^{a}	15.79±0.14^{a}	15.88±0.23^{a}	0.629±0.01^{a}	0.249±0.02^{a}
ABM+AA+SO	10.99±0.43^{a}	15.24±0.49^{a}	16.68±0.07^{c}	16.64±0.29^{a}	0.630±0.01^{a}	0.222±0.01^{a}

Values are expressed as mean ± SEM (n = 6 per group). Values with different letters in a column are significantly different at level $p < 0.05$. AA: Ascorbic acid, ABM: Abamectin, GSH: Reduced Glutathione, MDA: Malondialdehyde, SO: Sesame oil


Abamectin (ABM)
AA
SO
Oral
Male Wistar
Albino rats
Portal circulation
ABM
OS
SO
CYP
2E1
ROS
MDA
Lipid PXO
TNF
GSH
CAT
Caspase-3
p38MAPK
Apoptosis
Liver
Systemic veins
Systemic arteries
Brain
Abcb1a
Abcb1a
Synapse
GABA-A
R